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TRANSLOCATION AND DISTRIBUTION OF PICLORAM IN BEAN PLANTS ASSOCIATED WITH NASTIC MOVEMENTS

Charles P. P. Reid
Woodland Hurtt

JANUARY 1969

DEPARTMENT OF THE ARMY
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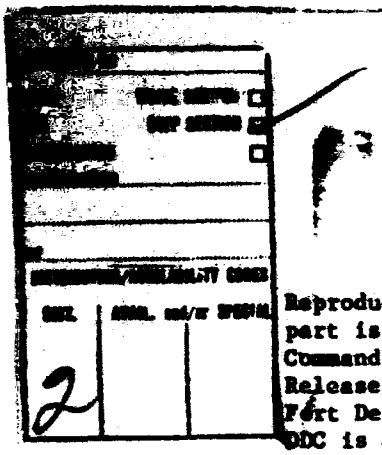

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TECHNICAL MANUSCRIPT 498

TRANSLOCATION AND DISTRIBUTION OF PICLORAM IN BEAN PLANTS ASSOCIATED WITH NASTIC MOVEMENTS

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Plant Physiology Division
PLANT SCIENCES LABORATORIES

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ABSTRACT

Nastic responses in bean plants (Phaseolus vulgaris var. Black Valentine) occur rapidly when very low concentrations of picloram (4-amino-3,5,6-trichloropicolinic acid) are applied to the root systems. Investigations were conducted to quantitatively determine the distribution of picloram in root-treated plants.

Sixty-seven micrograms of C^{14}-labeled picloram and 72 µg of unlabeled picloram in 300 ml of nutrient solution were applied to roots of 9-day-old bean plants growing in an environmental growth chamber at 24 C and 56% RH. Plants were removed from the treatment solution after uptake periods of 3, 6, and 11 hours. Sections were excised from ten locations on each plant and C^{14} content was determined by liquid scintillation techniques. As a qualitative indication of picloram mobility, additional plants were treated for 3 hours and autoradiographed.

The accumulation of picloram within various parts of the plant increased with treatment time. After 3 hours of picloram uptake, a curvature of 60 to 80 degrees from vertical in the upper 50 to 60 mm of stem corresponded to an accumulation in that part of the plant of 0.300 (second internode sample) to 1.065 (terminal bud sample) ng of picloram per mg fresh weight. The appearance of hyponasty in the trifoliolate leaflets after 6 hours of treatment corresponded to a picloram concentration in the leaf of 0.803 to 0.855 ng per mg fresh weight.

As evidenced by the C^{14} count data, the translocation of picloram from the roots to the apical part of the plant was very rapid. Picloram was preferentially accumulated in the terminal bud and first trifoliolate leaflets. Very little picloram was transported to the primary leaves and only at the longer treatment periods. Autoradiographs of plants treated for 3 hours showed qualitatively a similar distribution of C^{14} in the plant.

I. INTRODUCTION*

The herbicide picloram (4-amino-3,5,6-trichloropicolinic acid) has been characterized as comparable to 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid in absorption, translocation, and soil-leaching but as more effective on many broad-leaved plants.[5] Distortions in growing tissue, growth promotion in stem sections, and other morphogenetic effects caused by picloram have also been reported.[4,5,8]

In our investigations it has been observed that nastic responses in bean plants occur rapidly after the application of very low concentrations of picloram to the root systems. Symptoms are usually manifested in the upper portion of the stem and in the developing trifoliolate leaves. Responses are seldom detected in the primary leaves except in the case of high dosages. The rapid development of injury symptoms in stem and foliage has also been observed when picloram is applied foliarly.[6]

Because picloram evoked visible responses in such low concentrations, it was considered desirable to determine quantitatively how much picloram was actually distributed through the plant.

II. MATERIALS AND METHODS

Bean plants (Phaseolus vulgaris var. Black Valentine) were germinated in sand, transferred to aerated 0.5X Hoagland's nutrient solution, and grown in a controlled environment growth chamber. Uniform plants in which the first trifoliolate leaf had just opened (9 days old) were selected for use. Experiments were conducted under the following environmental conditions: 24±0.5 C, 56±4% relative humidity and a 16-hour photoperiod of 1,460 ft-c of illumination at plant-top level provided by a mixture of fluorescent and incandescent lighting.

Plants were treated by immersing the root systems in foil-covered pint jars containing 300 ml of 1×10^{-6} M picloram in 0.5X Hoagland's nutrient solution. The addition of 67.5 μg (0.287 μc) of C^{14}-labeled picloram (carboxyl labeled) to each jar resulted in a final treatment solution of 1.93×10^{-6} M picloram at pH 6.10. Aeration was stopped immediately before herbicide application. Plants used as controls were grown in nutrient solution (pH 6.10) containing no picloram.

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At periods of 3, 6, and 11 hours following the initiation of root treatment, plants were removed from the containers and sampled. Tissue sections were removed from 10 preselected locations on each plant. Each section was placed in a tared polyethylene scintillation vial and weighed to obtain fresh weight. Samples from treated and untreated plants were digested in the vials by an adaptation of a method of Mahin and Lofberg[9] used for animal tissue digestion. This consisted of adding 0.2 ml of 60% $HClO_4$ and 0.3 ml of 30% H_2O_2, mixing, sealing, and heating at 75 C for 90 minutes. After cooling to room temperature, 14.5 ml of toluene-Cellosolve scintillation solution (two parts toluene, one part 2-ethoxyethanol, and 6 g PPO/liter) were added to each vial. Background determinations were obtained from untreated plant samples.

Autoradiographs were made of two untreated plants and nine plants root-treated for 3 hours. Each plant was cut into several segments, pressed, and dried in a forced-draft oven at 90 C for the 1st hour and at 50 C for a subsequent 3 days. After drying, plants were processed in the usual manner for autoradiography.[3]

Tissue samples were counted 20 minutes each in a Nuclear-Chicago Model 6860 liquid scintillation spectrometer. To determine whether individual samples had a significant accumulation of radioactive isotope, the following equation[7] was used:

$$D = K \left[\frac{c_s}{t_s} + \frac{c_b}{t_b}\right]^{\frac{1}{2}}$$

in which D is the error of the difference between two counting determinations, K is a constant given in normal probability tables, c_s is the count rate of the sample, c_b is the count rate of the background, t_s is the length of time the sample was counted, and t_b is the length of time of the background count.

Sample count rates were corrected for efficiency by the external-standard ratio method and expressed on a counts/min per mg of fresh weight basis. This was then converted to total nanograms (ng) of C^{14}-labeled picloram and unlabeled picloram per milligram fresh weight. Assuming no preferential plant uptake of C^{14}-labeled over unlabeled picloram, the total quantity of picloram in each sample was calculated from the ratio of C^{14}-labeled picloram to unlabeled picloram previously determined in the treatment solution. The breakdown of the picloram molecule in the plant was presumed to be negligible because of the short time intervals involved and on the basis of studies with cotton reported by Meikle, Williams, and Redemann.[10] Curvature responses of stem and foliage were recorded throughout the treatment periods.

III. RESULTS AND DISCUSSION

An average curvature of 20 to 30 degrees from the vertical of the upper 50 to 60 mm of stems occurred within 2 hours after initiation of picloram treatment. At 3 hours, curvature had increased to 60 to 80 degrees. Upward curling of the first trifoliolate leaflets had started by 6 hours while stem curvature had increased to 70 to 90 degrees. At 11 hours after treatment, stem curvature remained at 70 to 90 degrees but upward curling of the leaflets was more pronounced.

The data on distribution of C^{14} in tissue sections removed from plants treated for 3, 6, and 11 hours are presented in Table 1. After 3 hours, all samples showed significant quantities of C^{14} except those taken from the primary leaves. Quantities of C^{14} at any particular sampling location increased with treatment time except in a few instances. The accumulation in total nanograms of picloram after the various treatment times is shown diagrammatically in Figure 1. Accumulation of picloram was greatest at the terminal bud followed in descending order of accumulation by first trifoliolate leaflets, stem sections, and primary leaves. As small a picloram concentration range as 0.300 ng/mg (second internode sample) to 1.065 ng/mg (terminal bud sample) effected a 60 to 80 degrees curvature in that part of the plant after 3 hours of uptake. Hyponasty in the trifoliolate leaflets observed after 6 hours of treatment corresponded to a concentration of picloram in the leaf ranging from 0.803 to 0.885 ng/mg.

A similar distribution of picloram in the apex and stem of bean plants was found when using foliar application of C^{14}-labeled picloram.[11] However, it appears the application of low concentrations of picloram to the root system is more effective than the application of higher dosages to the primary leaf. In our investigations, a total quantity of 140 µg of picloram applied to the roots resulted in an accumulation of 1.065 ng of picloram per mg of fresh weight in the terminal bud in 3 hours. Merkle and Davis[11] reported an accumulation of only 1.191 ng of picloram per mg of fresh weight in the apex of bean after 4 hours when 25,000 µg of picloram were applied to one primary leaf.

The average moisture content of 10 bean tissue samples was determined to be 93.2±4.6%. If the total amount of picloram detected in a tissue section was assumed to be distributed uniformly throughout the liquid phase of the tissue (93.2% of the fresh weight), an estimate of the molar concentration of picloram in a particular section could be calculated. For example, the molar concentration of picloram in the terminal bud after 3 hours of treatment can be calculated by converting 1.065 ng of picloram per mg fresh weight to 4.74×10^{-6} M. This indicates only a 2.4-fold concentration as compared with the original treatment solution, and serves only to emphasize the very low quantity of picloram involved in growth modification.

TABLE 1. SPECIFIC ACTIVITY[a] OF C^{14}-LABELED PICLORAM IN BEAN PLANT TISSUE AS A FUNCTION OF TREATMENT TIME

	Tissue Segment	Replicate 1	2	3	4	5	Mean
		3-Hour Treatment					
1	Terminal bud	4.61**[b]	6.84**	1.84**	5.72**	5.31**	4.864
2	2nd internode	2.11**	1.07**	0.82**	1.51**	1.34**	1.370
3	3rd internode	0.42**	0.99**	0.56**	0.35**	0.22*	0.512
4	4th internode	2.01**	1.25**	0.92**	0.73**	1.27**	1.236
5	Hypocotyl	1.62**	0.75**	0.97**	0.71**	0.50**	0.910
6	Trifoliolate leaf	2.63**	1.97**	1.67**	2.30**	2.48**	2.210
7	Trifoliolate leaf	3.98**	1.62**	1.38**	2.45**	2.04**	2.294
8	Primary leaf	0.06	0.00	0.09	0.08	0.10	0.066
9	Primary leaf	0.05	0.02	0.00	0.01	0.08	0.032
10	Primary leaf	0.16	0.00	0.12	0.11	0.09	0.096
		6-Hour Treatment					
1	Terminal bud	9.22**	14.60**	11.73**	13.76**	14.70**	12.802
2	2nd internode	0.82**	1.72**	3.33**	1.98**	2.41**	2.052
3	3rd internode	1.05**	0.84**	1.10**	1.16**	1.13**	1.056
4	4th internode	1.90**	1.73**	1.81**	1.41**	3.51**	2.072
5	Hypocotyl	0.72**	0.88**	0.71**	0.71**	0.57**	0.718
6	Trifoliolate leaf	2.54**	4.12**	4.63**	3.04**	5.88**	4.042
7	Trifoliolate leaf	1.49**	3.08**	4.07**	4.06**	5.63**	3.666
8	Primary leaf	0.09	0.25**	0.15	0.05	0.03**	0.168
9	Primary leaf	0.10	0.23	0.09	0.10	0.30**	0.164
10	Primary leaf	0.38**	0.14	0.17	0.18	0.19	0.212
		11-Hour Treatment					
1	Terminal bud	3.59**	20.33**				11.960
2	2nd internode	0.47	6.09**				3.280
3	3rd internoue	1.36**	1.99**				1.675
4	4th internode	1.07**	2.76**				1.915
5	Hypocotyl	0.79**	1.18**				0.985
6	Trifoliolate leaf	3.07**	5.68**				4.375
7	Trifoliolate leaf	2.28**	5.13**				3.705
8	Primary leaf	0.21*	0.40**				0.305
9	Primary leaf	0.05	0.32*				0.185
10	Primary leaf	0.29*	0.44**				0.365

a. Counts/min per mg fresh weight.
b. * = Significant at 5% level based on sample count rate.
** = Significant at 1% level based on sample count rate.

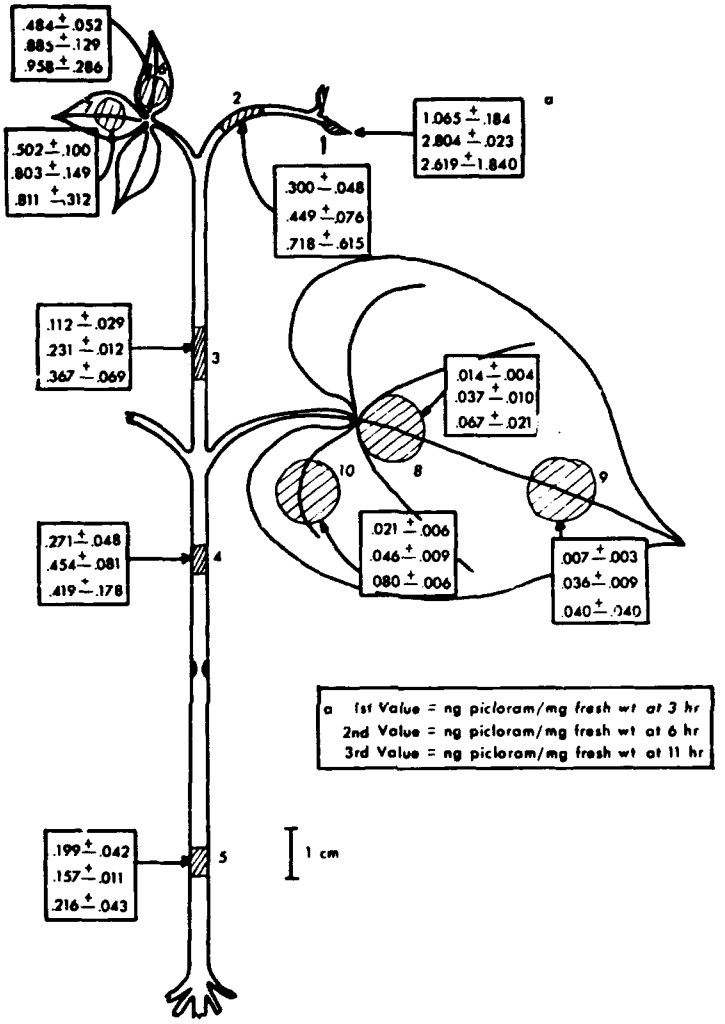

FIGURE 1. Diagrammatic Representation of Picloram Accumulation (ng/mg fresh wt) in Black Valentine Bean after 3, 6, and 11 Hours of Root Uptake. Mean values ± standard error of the mean are based on five replications for 3- and 6-hour uptake periods, and two replications for 11-hour uptake period.

The autoradiograph of the plants treated for 3 hours showed qualitatively a similar distribution of C^{14} as that revealed by the quantitative determination (Fig. 2).

FIGURE 2. Representative Autoradiograph Showing Distribution of C^{14}-Labeled Picloram after 3-Hour Root Uptake Period. A. Treated plant, B. autoradiograph.

Mention has been made in the literature[5] that the translocation characteristics of picloram are comparable to those of 2,4-D, but this is not necessarily the case. Our results with bean plants demonstrated that picloram is rapidly translocated out of the roots to areas of high metabolic activity. In contrast, other investigations using comparably larger quantities of 2,4-D in application to root systems, showed little 2,4-D translocation out of the roots of bean or Zebrina, and only slight amounts exported from the roots of barley and cotton.[1-3]

Although the rapid translocation of picloram from the roots to the apex of the plant might be viewed as xylem movement via the transpiration stream, it was noted that very little picloram was moved into the primary leaves where conceivably the majority of transpirational water loss would have occurred. While the data presented here are not sufficient to show whether picloram transport takes place via the phloem or xylem, they appear to support the findings of Merkle and Davis[11] which indicate that xylem transport is not necessarily the explanation for picloram transport. They demonstrated that moderate water stress (defined as a sap velocity 57% of control) had no significant effect on acropetal or basipetal translocation rate of picloram.

In this investigation, morphological aberrations in plant parts were caused by the presence of very low concentrations of picloram. Picloram was translocated rapidly from the roots to the apical part of the plant and preferentially accumulated in regions of higher metabolic activity. Relatively little picloram was transported to the primary leaves.

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13. ABSTRACT

Nastic responses in bean plants (Phaseolus vulgaris var. Black Valentine) occur rapidly when very low concentrations of picloram (4-amino-3,5,6-trichloro-picolinic acid) are applied to the root systems. Investigations were conducted to quantitatively determine the distribution of picloram in root-treated plants.

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